ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1991, p. 500–505
0066-4804/91/030500-06$02.00/0

Vol. 35, No. 3

SDZ MRL 953, a Novel Immunostimulatory Monosaccharidic Lipid A Analog with an Improved Therapeutic Window in Experimental Sepsis

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Received 29 May 1990/Accepted 23 November 1990

SDZ MRL 953, a new synthetic monosaccharidic lipid A, was investigated in vitro and in vivo for immunopharmacological activities. In experimental models of microbial infections, the compound was highly protective when it was administered prophylactically either once or three times to myelosuppressed or immunocompetent mice. The 50% effective doses of SDZ MRL 953 varied with the infectious agents and the route of its administration. In all cases, the 50% effective doses were about 10^3 times higher than those obtained with endotoxin from *Salmonella abortus equi*. SDZ MRL 953 was, however, less toxic than lipopolysaccharide by a factor of 10^4 to $>7 \times 10^5$ times in galactosamine-sensitized mice. The compound was also an effective inducer of tolerance to endotoxin. Hence, repeated dosing with the compound induced a transient resistance (≥1 week) to lethal challenges with endotoxin. In vitro, the compound was devoid of intrinsic antimicrobial activity, but it moderately induced the release of cytokines from monocytes and primed human neutrophils for the enhanced production of reactive oxygen metabolites in response to a soluble stimulus. The results presented here suggest that SDZ MRL 953 may be useful in a clinical setting for enhancing resistance to infections, particularly in patients undergoing myelosuppressive chemotherapy or irradiation, and for the prophylaxis of endotoxin shock.

Lipopolysaccharides (LPS) are common constituents of cell walls of gram-negative bacteria. They can cause a whole array of pathophysiological effects and are also the most powerful immunostimulants known. It is generally accepted that the lipid A moiety, the terminal acylated β(1–6)glucosamine disaccharide-1,4′-diphosphates of endotoxin, is responsible for immunopharmacological activity and induction of endotoxicity, such as changes in leukocyte count, disseminated intravascular coagulation, and multiorgan failure leading to irreversible shock and death (5, 21, 25).

Extensive studies have unsuccessfully addressed the possibility of harnessing the immunopharmacological activities of endotoxins by using various detoxifying approaches (17, 20). The elucidation of the correct structure of lipid A (8, 26) and the subsequent success in the total synthesis of biologically active lipid A and analogs (9–12, 27) have rekindled interest in the possibility of separating the immunostimulatory and toxic moieties of endotoxin. Efforts to identify beneficial immunostimulatory lipid A derivatives have concentrated on synthetic analogs representing both the nonreducing (10–12) and reducing sugar moieties, such as lipids X and Y (15, 27). Synthetic lipid A subunits of the nonreducing sugar moiety such as GLA-27 and GLA-60 were reported to activate B cells and macrophages and to induce release of mediators including gamma interferon and tumor necrosis factor (TNF) at nontoxic doses (for a review, see reference 5). The analogs were also found to be active in enhancing host resistance to microbial and viral infections in normal and myelosuppressed mice (5–7). Synthetic lipid X, the prototype reducing sugar moiety of lipid A, has been found to exhibit little (22) or none of the immunostimulatory activity (1, 14) previously attributed to the natural compound extracted from *Escherichia coli* mutant MN7 (3).

There is no unequivocal evidence in the literature supporting the notion that immunopharmacologically active synthetic lipid A subunit analogs have an improved therapeutic margin over that observed with intact endotoxin or lipid A in myelosuppressed infected animals. Our strategy to identify such lipid A analogs has been to compare highly purified synthetic analogs of the reducing sugar moiety of lipid A with endotoxin from *Salmonella abortus equi* for their capacity to induce shock in galactosamine-sensitized mice and their potencies to enhance nonspecific resistance to infections in mice. In addition, parallel in vitro investigations to evaluate the induction and release of cytokines from murine macrophages and the production of reactive oxygen metabolites from human neutrophils as possible modes of actions of the analogs were performed. This report shows that the synthetic lipid A analog SDZ MRL 953, 2-deoxy-3,4-bis-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamido]-1-*O*-phosphono-α-D-glucopyranose, expresses immunopharmacological activities similar to those of endotoxin but little of its toxicity. On the basis of the above selection criteria, it has, therefore, a broader therapeutic margin than endotoxin in experimental sepsis.

MATERIALS AND METHODS

Test substances. SDZ MRL 953 was synthesized at Sandoz Forschungsinstitut (SFI), Vienna, and purified by using a Sephadex LH 20 column to >99% purity, as assessed by reverse phase high-performance liquid chromatography (HPLC). LPS was a water-phenol extract of *S. abortus equi* (Sigma Chemical Co). Stock solutions of the compound and LPS were prepared by sonicating 1 mg in 10 μl of ethanol for about a minute, and then during further sonication for 10 min, sterile pyrogen-free isotonic glucose solution (5.4%) was added dropwise up to 1 ml. Under these conditions, SDZ MRL 953 formed a slightly opalescent solution that was

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stable at room temperature for weeks. For use, the stock solutions were diluted to the required concentrations with the appropriate media or buffers.

Animals. Female mice ($B_6D_2F_1$) weighing 18 to 20 g were obtained from Charles River Laboratories (Wiga, Sulzfeld, Federal Republic of Germany) and maintained on SFI's standard diet with water ad libitum.

Isolation of human neutrophils. Heparinized venous blood was obtained from healthy donors in accordance with the guidelines laid down by the ethical committee of the Intensiv-Blutbank, Allgemeines Krankenhaus, Vienna. Neutrophils were purified by Ficoll-Paque (Lymphoprep) gradient centrifugation followed by dextran sedimentation. Residual erythrocytes were lysed by the addition of 0.83% ammonium chloride solution. Purified neutrophils (>98%) were washed and resuspended in 0.1 M phosphate-buffered saline supplemented with 0.1% bovine serum albumin to 2×10^7 cells per ml.

Measurement of chemiluminescence. Production of reactive oxygen metabolites by the neutrophils was measured by a sensitive luminol-dependent chemiluminescence assay as described by Wymann et al. (31), by using a 1251 luminometer (LKB Wallac), which was equipped with a multivessel carousel transporter and held at a temperature of 37°C. Briefly, 10^6 neutrophils were added in a cuvette containing 10 μM luminol, 100 μM sodium azide, and 10 U of horseradish peroxidase per ml. Hydrogen peroxide production by the neutrophils was initiated with either SDZ MRL 953, LPS, or formyl-Met-Leu-Phe (f-MLP), and chemiluminescence resulting from the oxidation of luminol to chemically excited 3-aminophthalate was continuously recorded. Peak chemiluminescence readings were plotted against test substance concentrations.

For evaluating the priming effect of SDZ MRL 953 on neutrophils, the cells were preincubated with the appropriate amount of the compound at 37°C for about 2 or 60 min. Chemiluminescence in response to a suboptimal amount of f-MLP (10^{-7} M) was then measured as described above.

Cultivation of bone marrow-derived macrophages and induction of TNF. Bone marrow cells from $B_6D_2F_1$ mice were cultivated for 8 to 10 days in Teflon (E. I. du Pont de Nemours & Co., Inc.) bags in the presence of L-929 cell supernatant as a source for colony-stimulating factor 1 as previously described (22). For the induction of TNF by SDZ MRL 953 or by LPS, cells were plated out at 10^6 cells per well in 24-well Falcon plates in RPMI 1640 medium supplemented with 5% fetal calf serum. The macrophages were allowed to adhere at 37°C for 2 h, and the nonadherent cells were washed off. The adherent cells were cultivated overnight in the presence or absence of gamma interferon and were challenged with serial dilutions of the test substance for 4 h. Supernatants were collected, and the TNF content was assayed by its cytotoxicity on actinomycin D-treated L-929 cells (22).

Assessment of protective activity of SDZ MRL 953 against microbial challenge. *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* isolates were from the SFI culture collection. Each bacterial pathogen was cultivated overnight in tryptic soy broth (Difco Laboratories, Detroit, Mich.). *C. albicans* was grown in Sabouraud broth (Difco). The cultures were diluted in fresh broth to previously determined infective doses.

The protective effect of SDZ MRL 953 or LPS was assessed in systemically infected groups of 10 myelosuppressed or 10 normal $B_6D_2F_1$ female mice weighing approximately 20 g each. Mice were rendered neutropenic by a single subcutaneous (s.c.) injection of cyclophosphamide (200 mg/kg) on day −4 (i.e., before microbial challenge). On day −1, 10 mice were randomly divided into test groups and were treated either s.c., intraperitoneally (i.p.), or intravenously (i.v.) with serial dilutions of the compound dissolved in 0.1% ethanol-glucose solution in 0.2 ml volumes per mouse. On day zero, the animals were inoculated i.v. with the test microorganisms. The 50% effective doses (ED_{50}s) of the compound were calculated by probit analysis (16) from survival data 4 days postinoculation.

The protective activity of the compound was also evaluated in normal mice following either single or three consecutive prophylactic treatments. Microbial inoculation was performed by the i.p. route. The ED_{50}s were calculated as described above for myelosuppressed mice.

Assay of lethal toxicity in galactosamine-loaded mice. The sensitivity of galactosamine-loaded mice to SDZ MRL 953 or LPS was evaluated by the method of Galanos et al. (2). Briefly, groups of six mice (C57BL/6 or $B_6D_2F_1$) were simultaneously treated with galactosamine (400 mg/kg) i.p. and challenged with the compound or LPS by the i.v., i.p., or s.c. route. The 50% lethal dose (LD_{50}) was calculated by probit analysis (16). The ability of the compound to render experimental animals hyporesponsive to a lethal challenge with LPS was investigated in animals that had received the appropriate dose of the tolerance-inducing agent as a single or multiple prophylactic treatment.

RESULTS

Protection against bacterial and fungal infections in myelosuppressed mice. Cyclophosphamide is widely employed as a myelosuppressive agent because of its ability to induce leukopenia and its increased susceptibility to a variety of infections (23). To examine whether SDZ MRL 953 could modify the sensitivity of myelosuppressed mice to infections, the compound was administered to cyclophosphamide-treated mice 1 day prior to challenge with either *E. coli* (2×10^6 CFU per mouse), *P. aeruginosa* (6×10^4 CFU per mouse), or *S. aureus* (2×10^6 CFU per mouse) (Table 1). Most of the control mice usually succumbed to the infections within 24 h. The drug-pretreated animals were observed for 4 days and were considered long-term survivors if they lived beyond this period (19). The ED_{50}s obtained following i.p. pretreatments were about 1 mg/kg in all infections but were greater than 4 mg/kg when the compound was injected s.c. In experimental infection induced by *C. albicans* (2×10^4 CFU per mouse), i.p. pretreatment with SDZ MRL 953 also resulted in significant protection (ED_{50}, about 1 mg/kg). Enhanced resistance was also observed when the compound was injected either i.v. or s.c., but at an ED_{50} exceeding 5 mg/kg.

At this point, it had to be clarified whether three treatments with SDZ MRL 953 did not compromise the effect seen after a single prophylactic dose of the compound. The results indicate that a single dose of the compound was less effective than the same amount of the compound divided into 3 doses. For example, whereas single doses of SDZ MRL 953 became ineffective when the inoculum size of *S. aureus* was increased from 2×10^6 to 3×10^8 CFU per mouse, the same dose divided into three treatments on three consecutive days prior to microbial inoculation was highly protective (ED_{50}, 2.35 mg/kg [confidence limits, 1.6 to 6.3]). An increase in the protective effect of SDZ MRL 953 was also observed when the compound was administered three times prior to challenge with the low inoculum. For example, the

TABLE 1. Efficacy of single treatment with SDZ MRL 953 or LPS against microbial infections in cyclophosphamide-myelosuppressed mice[a]

Pathogen	CFU/mouse	Route and minimum effective dose (ED_{50})[b] to protect infected animals	
		SDZ MRL 953 (mg/kg)	LPS (μg/kg)
Pseudomonas aeruginosa ATCC 29511	6×10^4	i.p. 1.1 (0.8–1.4) i.v. 1.6 (1.1–2.3) s.c. 5.7 (2.2–9.3)	i.p. 1.9 (0.5–9.0) i.v. 2.1 (0.9–6.3) s.c. 3.1 (1.6–6.8)
Escherichia coli O1:K1	2×10^6	i.p. 1.0 (0.5–1.7) i.v. 1.4 (0.01–5.3) s.c. 4.5 (2.7–6.9)	i.p. 0.4 (0.0–3.6) i.v. 0.3 (0.1–0.6) s.c. 4.0 (2.3–6.6)
Staphylococcus aureus ATCC 10390	2×10^6	i.p. 0.9 (0.3–1.6) i.v. 1.0 (0.2–4.8) s.c. 4.1 (2.8–5.8)	i.p. 0.3 (0.1–0.8) i.v. 8.8 (1.1–4.6 × 10^5) s.c. 8.0 (3.3–20.8)
Candida albicans Δ24	2×10^4	i.p. 0.8 (0.6–1.2) i.v. >5 s.c. >20	i.p. 11.8 (5.9–19.7) i.v. 1.2 (0.2–3.7) s.c. 10.5 (4.9–22.2)

[a] Mice were rendered myelosuppressed by a single s.c. injection of cyclophosphamide (200 mg/kg) 4 days prior to i.v. inoculation of the indicated pathogen. SDZ MRL 953 or LPS was given as a single (i.p., i.v., or s.c.) injection 24 h prior to infection. ED_{50}s were calculated by probit analysis from survival data on day 4 postinfection.

[b] Values in parentheses show confidence limits from probit analysis.

ED_{50} of 0.8 mg/kg (confidence limits, 0.6 to 1.2) obtained from single doses of the analog against *C. albicans* (2×10^4 CFU per mouse; Table 1) was reduced to 0.16 mg/kg (confidence limits, 0.07 to 0.3) when 3 doses were used.

To achieve to a similar degree the pharmacological effects exerted by SDZ MRL 953, about 1,000-times-lower doses of LPS were required. For example, parenterally injected LPS effectively protected mice against challenges with *P. aeruginosa*, *E. coli*, *S. aureus*, or *C. albicans*, with ED_{50}s of between 0.3 to 12 μg/kg (Table 1). Again, the degree of the enhanced nonspecific immunity varied with the mode of LPS administration. Optimal protection was obtained by i.p. administration of LPS. As observed with SDZ MRL 953, single treatment with LPS was also ineffective in protecting mice if high inocula were used. However, the same amount of LPS divided into three treatments improved survival against a challenge with *S. aureus* (3×10^8 CFU per mouse) at ED_{50}s of 2.25 μg/kg (confidence limits, 1.7 to 2.8). Similarly, three consecutive administrations of LPS to mice prior to inoculation of *C. albicans* reduced the ED_{50}s obtained after single doses from 11.8 (confidence limits, 5.9 to 19.7) to 0.12 μg/kg (confidence limits, 0.04 to 0.24).

Protection against microbial infections in normal mice. SDZ MRL 953 also enhanced the nonspecific resistance of normal mice to infections with *P. aeruginosa* (2×10^7 CFU per mouse) or *S. aureus* (3×10^8 CFU per mouse). The compound protected against both infections at ED_{50}s of 2.34 (confidence limits, 1.4 to 3.9) and 1.1 mg/kg (confidence limits, 0.6 to 1.8), respectively. LPS was inactive at up to 5 mg/kg against *S. aureus* infection, but the protective activities of both agents were considerably improved by increasing the predosing schedules. For example, whereas single pretreatments with SDZ MRL 953 were protective in *S. aureus* infection with an ED_{50} of 2.34 mg/kg (confidence limits, 1.4 to 3.9), a similar protective effect was observed at an ED_{50} of only 0.04 mg/kg (confidence limits, 0.02 to 0.07) when the compound was administered on 3 consecutive days prior to infection. As in myelosuppressed mice (Table 1), the protective activities of both agents varied with the mode of administration.

Lethality of SDZ MRL 953 in galactosamine-loaded mice. Lethal toxicity of the compound and LPS was investigated in two inbred strains of mice, C57BL/6 and $B_6D_2F_1$, sensitized by galactosamine. Simultaneous i.p. administration of galactosamine (400 mg/kg) rendered both mouse strains more sensitive to LPS by a factor ranging from 7×10^3 to 3.8×10^5 (Table 2). The LD_{50} of SDZ MRL 953 administered by the various routes could not be calculated at doses higher than 125 mg/kg because of the limited solubility of the compound. Nevertheless, SDZ MRL 953 at up to 100 mg/kg was well tolerated by galactosamine-loaded mice. These findings indicate that SDZ MRL 953 is less toxic than LPS by a factor ranging from 10^4 to $>7 \times 10^5$.

Pyrogenicity in rabbits. Pyrogenicity studies were performed according to the U.S. Pharmacopeia (29) in hybrid rabbits (New Zealand White-Russian). The maximum nonpyrogenic and minimum pyrogenic doses of SDZ MRL 953 were 1 and 5 μg/kg, respectively, when the compound was administered i.v. Under similar experimental conditions, the nonpyrogenic dose of LPS ranged between 0.25 and 0.5 ng/kg. Hence, SDZ MRL 953 is less pyrogenic than LPS by a factor of 10,000.

Priming of blood neutrophils. Bacterial LPS potently prime mature neutrophils in vitro for an enhanced respiratory burst on exposure to soluble stimuli (4, 24). To explore whether SDZ MRL 953 is still endowed with the priming effect of LPS, blood neutrophils were preincubated in vitro with serial dilutions of the compound at 37°C for 2 or 60 min. The production of reactive oxygen metabolites in response to 10^{-7} mol of f-MLP per liter was then studied. Preincubation of the cells with SDZ MRL 953 readily increased the production of reactive oxygen metabolites in response to f-MLP, with the maximum effect occurring within 2 min of exposure of the cells to the compound (Fig. 1). The observed priming was dose dependent and peaked at about 10 μg/ml. Similar priming was observed with LPS, but only if the cells were preexposed to LPS for at least 1 h (data not shown).

Induction of TNF release by SDZ MRL 953. The ability of SDZ MRL 953 to induce murine bone marrow-derived macrophages to secrete TNF-α is shown in Table 3. SDZ

TABLE 2. Lethal toxicity of SDZ MRL 953 in galactosamine-loaded mice compared with that of LPS

Mouse	Sensitized by GalN	Application	SDZ MRL 953		LPS	
			LD_{50} (μg/kg)	Confidence limits	LD_{50} (μg/kg)	Confidence limits
C57BL/6	+	i.p.	>125,000		0.17	0.1–0.28
	+	i.v.	800	600–1,000	0.02	0.01–0.03
	+	s.c.	>125,000		7.4	5.5–9.3
$B_6D_2F_1$	+	i.p.	>125,000		0.27	0.0–1.1
	+	i.v.	1,300		0.08	0.01–0.2
	+	s.c.	>125,000		4.1	2.8–16.6
C57BL/6	−	i.p.	>125,000		10,600	8,360–13,080
	−	i.v.	> 50,000		7,500	
	−	s.c.	>125,000		53,410	
$B_6D_2F_1$	−	i.p.	>125,000		11,560	4,500–15,660
	−	i.v.	> 50,000		12,250	
	−	s.c.	>125,000		101,920	

MRL 953 at 10 μg/ml induced the secretion of TNF-α to the same degree as LPS at 10 ng/ml (factor, 1,000). When administered to C57BL/6 mice primed with heat-killed *Mycobacterium bovis* BCG, SDZ MRL 953 at up to 50 mg/kg was not as effective as LPS at 0.1 mg/kg in inducing TNF-α in mouse blood (data not shown). We conclude from the results presented here that, by inducing the production of moderate amounts of TNF, SDZ MRL 953 has a better chance for therapeutic application than LPS, which induces the production of high amounts of TNF and other cytokines that have been implicated in both increasing nonspecific host resistance and inducing endotoxin shock (18, 28). It follows, therefore, that therapeutic application of SDZ MRL 953 in individuals with chronic infection should not lead to unexpected toxic manifestations or to sensitization to other bacterial cell wall components.

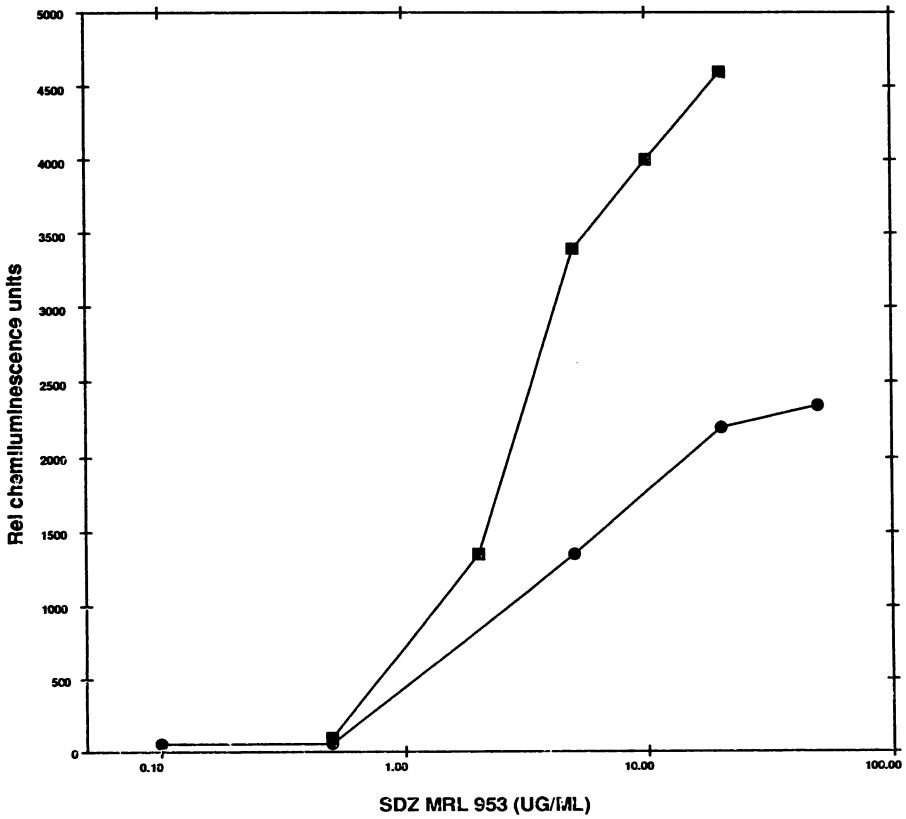

FIG. 1. Priming of human neutrophils by SDZ MRL 953 for enhanced f-MLP-stimulated respiratory burst. Neutrophils (10^6) were preincubated with SDZ MRL 953 at various concentrations for 2 min (■) or 1 h (●) at 37°C. Production of reactive oxygen metabolites by the cells in response to f-MLP (10^{-7} M) was measured as luminol-dependent chemiluminescence in a 1251 luminometer.

TABLE 3. Induction of TNF release by SDZ MRL 953 or LPS

Stimulus	Concn (μg/ml)	TNF (IU/ml)[a]	
		No gamma interferon	100 U of gamma interferon
SDZ MRL 953	10	158.3	424.4
	1	6.1	164.9
LPS	10	20.5	1,014.0
	1	6.8	85.7

[a] Adherent bone marrow-derived macrophages were cultivated overnight in the presence or absence of gamma interferon and then challenged with the stimulus for 4 h. The TNF released in the supernatants was assayed by cytotoxicity on actinomycin D-treated L-929 cells.

DISCUSSION

Endotoxins represent the most powerful immunostimulators known to date but are of limited clinical use because of their overt toxicity. The ability to harness the beneficial activity of LPS has eluded a number of investigators for years, and the interest in doing so has recently been rekindled by the finding that lipid A, the lipophilic terminus of LPS, is in fact endowed with most of the biological effects of LPS. Attempts to dissociate beneficial immunostimulatory properties from endotoxicity have resulted in the synthesis of a number of interesting lipid A partial structure analogs. Of these analogs, GLA-60, a lipophilic monosaccharide-4-phosphate mimicking the nonreducing sugar moiety of lipid A represents an achievement, in that it is still active against various infection models at about 10 μg per mouse without showing pyrogenicity in rabbits at >100 μg/kg (5, 13). However, this compound is still toxic in galactosamine-loaded mice at a dose of 1 μg per mouse (13). There is thus no convincing evidence now showing that GLA-60 or other lipid A analogs exhibit an improved therapeutic margin over LPS when their immunostimulatory activities are compared with either inherent or contaminating residual toxicities.

Our strategy to identify lipid A analogs which offer some potential for therapeutic use clinically has been to compare various synthetic analogs of the reducing sugar moiety for their efficacy in murine infection models with their endotoxic potential in galactosamine-sensitized mice. To avoid the problem of reproducibility of biological results that was experienced with synthetic lipid X (1, 14), we applied stringent criteria for the purification and solubilization of SDZ MRL 953 before the present study was performed. As with endotoxin, optimal protective activity of SDZ MRL 953 was observed only upon prophylactic administration 1 to 4 days before the mice were challenged with *E. coli*, *P. aeruginosa*, *S. aureus*, or *C. albicans*. The induced augmentation of nonspecific resistance was not equivalent in all the experimental models and varied with the time and route of drug administration. Similar variation was also observed in the activities of LPS used as an experimental standard. However, the variability in the protective activity of LPS was always wider than with SDZ MRL 953 in any given experiment. SDZ MRL 953 was about 10^4 to $>7 \times 10^5$ times less toxic than LPS in galactosamine-sensitized mice. For example, whereas i.p.- or s.c.-injected SDZ MRL 953 was not lethal in galactosamine-loaded mice at LD_{50}s of >125 mg/kg, LPS administered by the same route was lethal at LD_{50}s of 0.17 to 7.4 μg/kg. Hence, the compound was less toxic than LPS by a factor of 1.7×10^4 to $>7.4 \times 10^5$. Thus, the therapeutic indices of SDZ MRL 953 expressed, e.g., as the LD_{25}/ED_{75} were significantly improved over those of endotoxin and ranged from about 5 to >500, depending on the infection model and mode of administration.

There is a clinical concern that systemic administration of an apparently nontoxic analog of LPS might still elicit significant toxic side effects because of mechanisms of sensitization to other bacterial components. The results presented here suggest that SDZ MRL 953 does not induce high levels of putative mediators of endotoxin shock either in normal, galactosamine-sensitized, or BCG-primed mice. Moreover, repeated dosing with SDZ MRL 953 rendered the animals hyporesponsive to endotoxin (data not shown). For example, three consecutive injections of the compound resulted in increasing resistance to an otherwise lethal endotoxin challenge. The induced tolerance lasted for at least 1 week and did not negate its immunostimulatory activity. The underlying mechanisms of this intriguing effect are poorly understood at this time (28, 30).

The mechanisms by which SDZ MRL 953 and LPS enhanced nonspecific immunity in myelosuppressed and normal mice are under investigation and are thought to be mediated, in part, by the release of cytokines and an accelerated rate of hematopoietic recovery. In vitro, SDZ MRL 953 exerted no antibacterial effect and moderately stimulated mouse macrophages to release cytokines such as interleukin-6, interleukin-8, TNF, and colony-stimulating activity (14a) at relatively high concentrations. The compound also directly primed human neutrophils for an enhanced respiratory burst in vitro. Since it is known that virtually all the responses of an animal host to LPS are mediated by the action of macrophage-derived cytokines (18, 28, 30), the modest amounts of these factors which can be induced by SDZ MRL 953 would be expected to either prime mature phagocytic cells or stimulate hematopoiesis in vivo. That this is a distinct possibility was recently supported by the observations of Hildebrandt et al. (4a) showing that SDZ MRL 953 stimulated an early hematopoeitic recovery from myelosuppressive chemotherapy by expanding granulocyte-macrophage colony-stimulating factor-sensitive progenitor cells in the bone marrow that are sensitive to granulocyte-macrophage colony-stimulating factor.

In summary, the results presented here suggest that SDZ MRL 953 may have a therapeutic potential for enhancing resistance to infections during myelosuppressive therapy. It is expected that it will reduce the risk of endotoxic shock in traumatized patients or individuals with other risk factors for septicemia whenever prophylactic treatment is possible.

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